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☐ *Additional inventors are being named on the ____ separately numbered sheets attached hereto.*

TITLE OF THE INVENTION (280 characters max)

NUP153 DOMAIN-SPECIFIC REAGENTS

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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government

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I hereby certify that this patent application in the name of Katharine Ullman and Jin Liu for NUP153 DOMAIN-SPECIFIC REAGENTS, together with the drawings, and a Credit card form for the amount of $80.00 are being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. § 1.10 on the date indicated above in an envelope addressed to Box Patent Application, Assistant Commissioner for Patents, Washington, D.C. 20231.

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UNITED STATES PROVISIONAL PATENT APPLICATION

of

Katharine Ullman

Jin Liu

for

NUP153 DOMAIN-SPECIFIC REAGENTS

NUP153 DOMAIN-SPECIFIC REAGENTS

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to compounds for regulating mitotic events. More specifically, the present invention relates to agents capable of arresting nuclear envelope breakdown and assays for use in discovering compounds capable of arresting the cell-cycle.

2. Summary of the Invention

The compounds and method of the present invention have been developed in response to the present state of the art, and in particular, in response to the problems and needs in the art that have not yet been fully solved by currently available mitosis-inhibiting compounds and assays for the discovery of such compounds. Thus, the present invention provides compounds for arresting nuclear envelope breakdown and assays for screening for compounds with such properties.

We have demonstrated that *Xenopus laevis* (frog) egg extract provides a useful system in which to test for inhibitors of nuclear envelope breakdown, a process that normally ensues during the mitotic phase of the cell cycle. In particular, we have adapted a well-characterized system in which cell cycle events can be recapitulated *in vitro*. By monitoring nuclear envelope breakdown, we have found that a recombinant protein encompassing a specific domain of the pore protein Nup153 arrests the normal progression of mitotic events.

When the nucleus forms and breaks apart during each cell cycle, a vast array of structures must be assembled and disassembled in a coordinated fashion. Our studies have focused on the nuclear envelope and how the processes of recruitment and disassembly of the nuclear envelope are influenced by a component of the nuclear pore complex, Nup153. Nup153 is localized to the nuclear basket of the pore complex and has been implicated in specific routes of export and import. Nup153 was previously determined to associate with the chromosomal surface at an early time point during nuclear envelope assembly. Such early presence of the Nup153 protein suggests that it may play a role in recruiting membranes which will later form the nuclear envelope and/or in coordinating the process of nuclear pore complex formation.

Nup153 is composed of three major domains: a unique N terminus, a zinc finger region, and a C-terminal region containing FG repeats characteristic of many nucleoporins. We have used Nup153 domain-specific reagents to probe for roles in nuclear assembly and disassembly events reconstituted in *Xenopus* egg extract. This approach has provided evidence that Nup153 plays a role in these processes. Nup153 has previously been demonstrated to be dispensable for formation of a functional nuclear envelope, suggesting that a role at this stage is not essential, but may only be required under specific circumstances, or may normally serve to increase the overall efficiency of assembly. Interactions involving Nup153 at the time of disassembly are distinct from those that are important to nuclear assembly.

Membrane vesiculation has been proposed as a key mechanism in early studies of nuclear envelope breakdown/disassembly. This suggestion is a controversial one, however. In contrast, different lines of experiments have converged on the importance of

a collection of events that include breakdown of the nuclear lamina (Gerace *et al.*, 1978), dynein-mediated tearing of the nuclear envelope (Salina *et al.*, 2002; Beaudouin *et al.*, 2002), and the appearance of integral inner nuclear membrane proteins in the endoplasmic reticulum (Yang *et al.*, 1997; Ellenberg *et al.*, 1997). We have found an unexpected role for Nup153, a nuclear pore complex protein localized primarily at the nuclear face of the pore. Specifically, Nup153 interacts with coatomer proteins previously characterized in the context of vesicular trafficking. Using specific antibodies to perturb coatomer complex function during nuclear disassembly, we have demonstrated that coat proteins do play an important role during nuclear envelope breakdown. These results point to new participants in the process of nuclear disassembly and suggest that a coatomer-mediated step is one of multiple mechanisms that are integrated to accomplish breakdown of the nuclear envelope.

Nuclear envelope breakdown has not hitherto been the target of functional screens for cell cycle inhibitors, in part because little is known about the regulation of this event. Using this assay, an interesting interaction has been identified to target for inhibition. Specifically, a region was located within the pore protein Nup153 which helps to recruit machinery needed to disassemble the nuclear envelope. Screens may be used to identify molecules, including small molecules, which interfere with this interaction. Any such interfering molecules would be predicted to arrest the cell cycle. Such inhibitory molecules may trigger a cell-cycle checkpoint in such a way that certain cancer cells respond inappropriately, and as a consequence of continued proliferation in the face of abnormal nuclear division, are susceptible to cell death. Similarly, inhibitors may be found which simply arrest the cell cycle of both cancerous and normal cells, and which

acts specifically such that secondary effects are minimal, thus rendering cancer cells vulnerable to cell death due to their rapid proliferation. This approach may be adapted for high-throughput analysis for use in discovery of molecules which arrest the cell cycle. Use of this novel approach may lead to the characterization of a novel class of cell-cycle inhibitors.

Different regions of Nup153 of *Xenopus laevis* were produced in bacteria and purified by affinity chromatography. They were then used to raise antisera directed against these specific domains of Nup153. These domain-specific reagents will be useful in assays probing the function of Nup153, and for screening for inhibitors of pathways having therapeutic relevance. These reagents allow a systematic approach to inhibiting Nup153. Antibodies directed against different regions of Nup153 bind to distinct features of the nuclear pore architecture, implying that the domain of Nup153 targeted may have distinct functional outcomes. In addition, specific recombinant Nup153 protein fragments show blocking activity in an *in vitro* assay that monitors nuclear envelope breakdown. The availability of this panel of reagents will allow systematic blocking of Nup153 functions, allowing characterization of the functions of Nup153 and its interactions.

These and other features and advantages of the present invention will become more fully apparent from the following description, or may be learned by the practice of the invention as set forth hereinafter.

BRIEF DESCRIPTION OF THE DRAWINGS

In order that the manner in which the above-recited and other features and advantages of the invention are obtained will be readily understood, a more particular description of the invention briefly described above will be rendered by reference to specific embodiments thereof which are illustrated in the appended drawings. Understanding that these drawings depict only typical embodiments of the invention and are not therefore to be considered to be limiting of its scope, the invention will be described and explained with additional specificity and detail through the use of the accompanying drawings in which:

Figure 1 is a schematic illustration of initial steps in a system for recapitulating nuclear structure and function *in vitro*;

Figure 2 is a schematic illustration of later steps in a system for recapitulating nuclear structure and function *in vitro;*

Figure 3 contains illustrations of the Nup153 nuclear pore protein determined to have a role in nuclear disassembly and a domain-specific recombinant protein containing the central zinc-finger region of Nup153;

Figure 4 illustrates,the failure of nuclear envelope breakdown in the presence of the recombinant protein with the Nup153 zinc-finger domain of Figure 3;

Figure 5 shows blots of Nup153-specific antibodies; and

Figure 6 illustrates the failure of nuclear envelope breakdown in the presence of the Nup153-specific antibodies of Figure 5.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The presently preferred embodiments of the present invention may be best understood by reference to the drawings. It will be readily understood that the components of the present invention, as generally described and illustrated in the figures herein, could be arranged and designed in a wide variety of different configurations. Thus, the following more detailed description of the embodiments of the compounds, system, and method of the present invention, as represented in Figures 1 through 6, is not intended to limit the scope of the invention, as claimed, but is merely representative of presently preferred embodiments of the invention.

Nuclear envelope breakdown is a critical component of the cell cycle in higher eukaryotes. Although integral membrane proteins associated with the nuclear membrane have been observed to disperse into the endoplasmic reticulum at mitosis, the mechanisms involved in this reorganization have not been examined. Here, we report a role for the COPI coatomer complex in nuclear envelope breakdown, implicating vesiculation as an important step. Moreover, we have found that the nuclear pore protein, Nup153, plays a critical role in directing COPI to the nuclear membrane at mitosis. These results provide insight into how keys steps in nuclear division are orchestrated.

Introduction

At interphase, eukaryotic genomic DNA is enclosed by two concentric membrane bilayers termed the nuclear envelope. During open mitosis, which is characteristic of most metazoan cells, nuclear envelope breakdown allows for critical connections to be made between the mitotic spindle apparatus and condensed chromosomes. Ultimately, this process results in the accurate inheritance of both nuclear envelope components and

genomic DNA. Recent progress has led to a unified view of the mechanisms involved in disintegration of the nuclear envelope [1-3]. Namely, key events currently appear to be disassembly of the nuclear lamina meshwork that underlies the inner nuclear membrane, creation of a tear in the nuclear envelope due to microtubule-dependent tension exerted on the nuclear envelope [4, 5], and an increase in lateral mobility of integral membrane proteins allowing equilibration between the nuclear envelope and the endoplasmic reticulum and resulting in the loss of identity between these membrane populations [6, 7]. The possibility of specialized microdomains within the mitotic endoplasmic reticulum has also been suggested [7, 8]. However, separate NE-specific vesicle populations, although proposed in earlier studies, are not thought to be the norm at mitosis. Indeed, recent models, which have emerged from comprehensive integration of data, have discounted vesiculation as a physiological component of nuclear envelope breakdown [1-3, 8].

Inner and outer nuclear membranes are bridged by macromolecular nuclear pore complexes that each serve as a conduit for traffic between the nucleus and cytoplasm. Being situated in this regular array in close proximity to the nuclear membrane uniquely positions nuclear pore proteins (nucleoporins) to help execute steps in the process of nuclear envelope breakdown. However, outside the context of facilitating nucleocytoplasmic trafficking of cell cycle factors, a role for nucleoporins in nuclear envelope disassembly has not been explored. We chose to probe the function of Nup153 in nuclear envelope disassembly using a battery of domain specific reagents. Through this analysis, we have discovered a critical role for this pore protein in nuclear envelope breakdown. To better understand this novel role, we identified proteins that interact with

a relevant domain within Nup153. This led to the surprising finding of a partnership between Nup153 and the COPI complex. This coatomer complex has been previously characterized in the context of bud formation in vesicular trafficking within the Golgi and between Golgi and ER [9]. Here, we find that perturbing the function of the COPI complex leads to impaired nuclear envelope disassembly. These results lend insight into key players at this important stage of the cell cycle. Moreover, our data links the machinery of vesicular trafficking to nuclear envelope breakdown, indicating a greater complexity to this process than currently appreciated, but giving new clues about the mechanisms that are involved.

Results

The nucleoporin Nup153 plays a critical role in nuclear envelope breakdown.

To investigate whether Nup153 participates in nuclear envelope breakdown, we examined whether recombinant fragments of Nup153 exert dominant negative affects on this process *in vitro*. We used cell-free extracts derived from *Xenopus* eggs to form synthetic nuclei around sperm chromatin. At the outset, cycloheximide was included to prevent synthesis of cyclin, thereby arresting these extracts in interphase. After nuclei were assembled, the cell cycle block was overcome by addition of a recombinant form of stabilized cyclin B. These conditions are well established to recapitulate progression into mitosis and, as expected, 20 to 40 minutes after addition of cyclin, the nuclei synchronously exhibited hallmark features of mitosis, including chromatin condensation and the disappearance of a continuous nuclear membrane. When the reaction contained a region derived from the unique N-terminal domain of Nup153 (amino acids 436-636), nuclear envelope breakdown proceeded without any marked alterations. A control GST

fusion protein likewise had no affect. However, when a fragment encompassing the central zinc finger domain of Nup153 was included in the reaction, a striking inhibition of nuclear envelope breakdown was apparent. The affect of the zinc finger was not due to selective stability of this fragment, as equivalent levels of recombinant protein were detected in each reaction by immunoblot following the last time point.

Although it was clear from this result that the Nup153 zinc finger region could interfere with a step in nuclear disassembly, such a dominant negative approach leaves open the question of whether the inhibitory protein fragment exerts an affect by cross-interference with another protein that shares homology. To be certain that the block imposed by this fragment was due to interference with a function attributable to Nup153, we used antibodies that specifically recognize Nup153, as assessed by both immunoblot analysis and immunoprecipitation. When these antibodies were included in the nuclear disassembly assay, they too prevented the normal progression of events: the nuclear membrane stayed largely intact. This was true for independently raised antibodies as well, providing evidence that Nup153 plays a critical role during nuclear envelope breakdown. Of note, NLS mediated nuclear import took place as usual in the presence of these antibodies, indicating that there was no general impediment to movement through the nuclear pore complex, consistent with results with similar antibodies injected into oocytes [10].

Nup153 associates with members of the COPI coatomer complex. The zinc finger region of Nup153 is known to interact with RanGDP as well as with certain transport receptors [11-13]. Given the critical nature of nucleocytoplasmic trafficking during cell cycle progression [14], one possibility is that the factor that is saturated or

otherwise interfered with by the Nup153-specific reagents has a role in an import or export event. Nup153 also plays more structural roles, as is illustrated by its interaction with lamins [15] and its importance to nuclear pore basket formation and anchoring [16]. As such, Nup153 has potential to provide a scaffold for bringing together different proteins needed for progression of nuclear envelope breakdown. To determine how the zinc finger domain exerts a dominant negative effect on nuclear envelope breakdown, we used an unbiased approach to identify proteins that can interact with this region. Toward this end, recombinant zinc finger protein was immobilized and incubated in *Xenopus* egg extract. We found four major bands by silver stain analysis of the proteins that remained bound through this procedure but were absent in a parallel preparation with GST immobilized on the pull-down matrix. Mass spectrometry analysis revealed that among the proteins found in association with the Nup153 zinc finger region were three members of the COPI coatomer complex: β, β' and α. Other members of this complex would not have been detected as we had concentrated our efforts on proteins that were larger than 55 kDa due to the interference of the fusion protein in the analysis of smaller proteins. The surprising identification of COPI components in association with the zinc finger domain of Nup153 was confirmed by immunoblot analysis of similarly prepared samples.

To determine the significance of this interaction, we wanted to test for a function of the COPI complex in nuclear disassembly. Commercial antibodies, although cross-reactive on immunoblots, did not work well in immunoprecipitations from egg extract (data not shown), indicating that their recognition of native *Xenopus* β-coatomer was inefficient. We therefore raised an antibody using a *Xenopus* based peptide that corresponds to a peptide used successfully to generate antibodies that recognize native

human β-coatomer. The resulting antibodies are highly specific when used as a probe in an immunoblot of the complex mixture of proteins found in *Xenopus* egg extract and are able to recognize native protein as determined by their efficiency in an immunoprecipitation of β-coatomer from egg extract. With this reagent in hand, we could now definitively address the role of this coatomer protein in nuclear envelope disassembly. When the nuclear assembly/disassembly system was challenged with antibodies directed against β-coatomer, clear prevention of nuclear envelope breakdown was observed. This leads us to conclude that β-coatomer, and likely the entire COPI complex, plays a critical role in disassembly of the nuclear envelope.

COPI is recruited to nuclear membranes in a manner facilitated by Nup153 and ARF-dependent. Although recruitment of COPI to the nuclear envelope is suggested by our findings that COPI plays a role at a specific stage of nuclear envelope breakdown, to our knowledge such a localization has not been reported. In an intact cell, localization at the nuclear envelope may be obscured by the relatively greater amounts of Golgi membranes present. It is also probable that such an interaction is transient and occurs only within a specific window of the cell cycle. We therefore tested the prediction that COPI associates with the nuclear envelope using nuclei assembled in the egg extract system and enriched away from other membrane populations through a sucrose cushion. We also probed for the presence of β-coatomer at different times relative to the shift from interphase to mitosis. Before cyclin is added, no clear signal was obtained for β-coatomer at the nuclear envelope; detection of DNA (data not shown) as well as nuclear pore proteins confirms the presence of nuclei. In contrast, after mitotic signals are

triggered by cyclin addition, nuclear rim staining for β-coatomer was observed. This becomes noticeable at 40 minutes post-cyclin addition and persists at 60 minutes in the nuclei that have not yet undergone nuclear envelope breakdown. The specificity of this detection was confirmed by blocking reactivity of the antibodies with the immunogenic peptide, which resulted in a corresponding decrease in the nuclear rim signal. Pre-immune antisera was also tested and found to display no background signal.

This newly-observed localization of β-coatomer is consistent with a role for the COPI complex at this stage of nuclear division. To look specifically at the role that Nup153 plays with respect to COPI, we examined whether the zinc finger region of Nup153 alters the localization pattern of COPI at the nuclear envelope. We found that samples that contained the zinc finger domain and therefore arrested at a stage prior to NEB, also did not have significant recruitment of β-coatomer to the nuclear envelope. Thus, this region of Nup153 likely exerts a dominant negative affect on NEB by preventing recruitment of the COPI complex. It remains possible that Nup153 plays other important roles at this step via additional mechanisms such as transport regulation. Indeed, by participating at more than one level in nuclear envelope breakdown, Nup153 may help to coordinate the different mechanisms that underlie nuclear division.

As a final test of the proposed role for COPI in nuclear envelope breakdown, we used a peptide derived from the small GTPase ARF1 to interfere with ARF-mediated coatomer recruitment. This peptide has been found to interfere with ARF1 function and, in turn, impedes coatomer recruitment that is typically ARF dependent [17]. When this peptide was included in the assembly/disassembly reaction, nuclear envelope breakdown was impaired, with only a percentage of the nuclei remaining intact minutes after cyclin

addition. In contrast, a peptide composed of the reverse sequence had only minimal affects on nuclear disassembly. These results indicate that nuclear envelope breakdown requires the participation of ARF. Thus, the machinery involved in COPI recruitment to the nuclear membrane consists of a prototypic member of the well-characterized coat forming cycle as well as a new and unexpected modulator of this process.

Discussion

Nucleoporins: new players in nuclear envelope breakdown. It is increasingly clear that the nuclear pore undergoes global changes early in mitosis and indeed may serve a critical recruiting role for machinery involved in nuclear envelope breakdown. One line of evidence that points toward alteration of the nuclear pore complex early in the process of nuclear envelope breakdown is the shift in the nucleocytoplasmic diffusion cut-off that has been observed at this juncture [18]. The role of individual pore proteins as critical factors in recruiting disassembly machinery is illustrated by the functionally important interaction reported in this study between Nup153 and COPI. The NPC has also been proposed to be an anchor point for proteins involved in the connection between the nuclear envelope and the dynein-dynactin complex [1, 4, 5]. In this case, a specific nucleoporin has not been implicated, but certainly further exploration of the interface between components of the nuclear pore and proteins involved at various levels in nuclear envelope breakdown is likely to provide insight into how the process of nuclear envelope breakdown is orchestrated.

Nup153, in particular, is poised to participate in coordinating events that are important to NEB because it interacts both with the nuclear lamina [15] and the COPI coatomer complex. However, one reason that a role for Nup153 in COPI recruitment was

surprising is the localization of Nup153 predominantly on the nuclear basket structure of the nuclear pore. Although this vantage point does not seem ideally suited to the recruitment of a complex that is primarily cytoplasmic, many scenarios can be envisioned that would explain this apparent dilemma over accessibility. The coatomer complex may gain access through traditional import mechanisms, however the time-frame of detection of coatomer recruitment to the nuclear envelope suggests that mitotic conditions may have already altered nucleocytoplasmic trafficking. In fact, mitosis-specific alterations in nucleocytoplasmic flux may be permissive for an interaction between Nup153 and coatomer. It is also noteworthy that Nup153, although considered a component of the nuclear pore basket, appears to be exposed on the cytoplasmic side of the pore as well [11] (Fahrenkrog). The zinc-finger domain, however, is not accessible on the cytoplasmic face of the pore, either because it is anchored at the pore basket or because it is masked on the cytoplasmic face (Fahrenkrog). In either case, alterations early in mitosis could lead to exposure of the Zn finger domain on the cytoplasmic face of the pore and the opportunity for direct access with the COPI complex. Finally, large-scale perforations in the nuclear envelope, which occur in mitosis [4, 5], suggest that, at least after these initial events, there is ample opportunity for cytoplasmic components to access nuclear binding partners. These and other possibilities are not mutually exclusive and illustrate the range of mechanisms that could circumvent the apparent constraints on the surprising partnership between Nup153 and coatomer proteins.

The specific domain of Nup153 that has been identified in this study to participate in nuclear envelope breakdown also gives rise to interesting implications. Such a zinc finger like region is found in one additional vertebrate pore protein, Nup358. The

Nup153-specific antibodies used here directly demonstrate that Nup153 is important for NEB, but this does not rule out an important, perhaps cooperative, role for Nup358 and its Zn finger region in particular. Indeed, we have found that the Zn finger region within Nup358 can associate with the COPI complex although with an apparent lower affinity. Further studies to probe the functional significance of this interaction will be interesting. It is also notable that such a Zn finger like region has not been found in the repertoire of *S. cerevisae* pore proteins [19], raising the provocative question of whether inclusion of this domain module evolved as part of the vertebrate pore due to a critical role in disassembly of the nuclear envelope during open mitosis.

Integrating vesiculation into a working model of nuclear envelope breakdown. Recent evidence has pointed toward a thorough dispersal of integral nuclear membrane proteins into the ER [6, 7]. Since vesicles did not appear to be an end-point of nuclear envelope break-down in these studies and because lateral movement between the nuclear envelope and ER membranes could explain the observed absorption of NE into the ER, a role for vesiculation has been largely discounted in recent models of nuclear envelope breakdown [2, 8]. However, a role for vesicle budding in nuclear disassembly does not have to go hand-in-hand with segregation of NE and ER components. At least under some circumstances, vesicles formed from the nuclear envelope by a classical budding pathway could in fact be an additional mechanism for dispersing the contents of the NE into the ER via fusion of the NE-derived vesicles with the ER (*see* [6]).

Why would two overlapping mechanisms exist for dispersion of NE components into the ER? While it would not be surprising to have redundant mechanisms to ensure the efficiency of this process, it is also possible that lateral diffusion and budding/fusion

direct distinct populations of NE components to the ER. In this respect, the role of a pore protein in facilitating COPI recruitment suggests that vesicles may form locally near the nuclear pores and potentially could be enriched in pore membrane itself. In certain cell types, or under particular circumstances, fusion with the ER may be limiting and a population of specialized vesicles may result instead. Perhaps when the number of pores is higher, more vesicles form and a subset of these remain in vesicle form rather than fusing with and dispersing into the ER. Such a scenario would reconcile data that supports a role for distinct vesicle populations during nuclear reassembly [20-22], but this remains to be addressed experimentally.

Parallels between the nuclear envelope and the Golgi at mitosis. Breakdown of the Golgi apparatus, like the nuclear envelope, appears to be the result of multiple mechanisms. Although some aspects of this process are controversial, one common theme in the current views of Golgi disassembly is that COPII-mediated ER to Golgi traffic is blocked at mitosis [23] while COPI mediated budding from the Golgi continues or even escalates during the initial stages of mitosis [24] (other). Our results point toward COPI playing an analogous role in facilitating nuclear envelope dispersal. This new angle on nuclear envelope breakdown does not preclude a role for additional mechanisms. Certainly, in the case of the Golgi apparatus, other mechanisms are thought to contribute to its disassembly at mitosis [25]. Indeed, larger scale reorganization, such as breakdown of the Golgi ribbon or tearing of the nuclear envelope, may be prerequisites for remodeling these membrane structures through vesiculation. In the case of nuclear envelope breakdown, perforation in the nuclear envelope and/or other events such as alterations at the pore may be critical for efficient *recruitment* of the COPI complex to

the inner membrane of the nuclear envelope (see above) as well as for other independent and/or cooperative mechanisms.

Consistent with these parallels between Golgi disassembly and nuclear envelope disassembly, these organelle membranes also share specific aspects of their biogenesis machinery. Several distinct stages of nuclear envelope assembly have been identified and molecular requirements are beginning to be understood [26]. Interestingly, nuclear envelope closure requires the AAA-ATPase, p97, and its partners Npl4/Ufd1 [27]. Then, during nuclear envelope growth, p97 is again required but now in conjunction with a different partner, p47 [27]. p97, along with p47, has been previously implicated in formation of Golgi and transitional ER following mitosis [28-30].

In contrast to this recently recognized corollary between nuclear envelope and Golgi assembly, past studies focused on the role of ARF1 had led to the conclusion that this small GTPase, which plays a role in bridging COPI-membrane interactions, does not have a critical role in nuclear envelope assembly or breakdown [31]. In these studies, ARF proteins were depleted from *Xenopus* egg extract using size chromatography and the resulting extract was used to make nuclei and consequently induce mitotic breakdown. By immunoblot blot analysis, ARF was found to be 97-100% depleted, yet nuclei that had been formed in this extract responded normally to mitotic signals. Although depletion appeared to be efficient in this study, this type of approach can be complicated by the biological activity of certain proteins even at extremely low concentration. In this instance, this is underscored by early studies of mitotic extracts in which an overall 10-fold dilution delayed complete nuclear breakdown only two-fold [32]. Given the clear results we have obtained using an alternative strategy to interfere with ARF function, a

peptide inhibitor, it seems likely that in these earlier studies a small population of this protein remained in the depleted extract (or membrane fraction) at levels adequate to promote nuclear envelope breakdown.

Cell cycle control of NEB: This and other recent studies have further highlighted that, far from being a passive process, nuclear envelope breakdown relies on a critical series of activities. These events may well be regulated as part of the intricate network of feedback that occurs during the cell cycle. With the identification here of key players in nuclear envelope breakdown, future experiments can now focus on how these interactions are regulated. An intriguing possibility is that local concentration of the small GTPase Ran, known to have a central role in nuclear assembly [33, 34] and also previously found to interact with the Zn finger region of Nup153 [11], modulates accessibility of this domain to COPI. Future studies designed to unravel this question will provide new insight into cell cycle regulation and the coordination of mitotic events. In addition, the efficiency with which the Zn finger region of Nup153 arrests nuclear envelope breakdown suggests that this step in the cell cycle is exquisitely vulnerable and could be targeted to discover novel and potentially clinically useful inhibitors of cell division.

Legends

1. A specific domain of Nup153 exerts a dominant negative effect on nuclear envelope breakdown. diagram, coom., IF, immunoblot
2. Antibodies specific to Nup153 prevent nuclear envelope breakdown. IF, west
3. ZnF interacts with COPI complex. Silver, ms sum., west on pull-down, IP
4. Characterization of anti-xCOP Anti-COP Ab inhib. NEB; IF, immunoblot
5. ARF pep or BFA

6. COP is recruited to NE/disrupted by ZnF model

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The present invention may be embodied in other specific forms without departing from its structures, methods, or other essential characteristics as broadly described herein and claimed hereinafter. The described embodiments are to be considered in all respects only as illustrative, and not restrictive.

ABSTRACT OF THE INVENTION

The present invention relates to compounds for regulating mitotic events. More specifically, the present invention relates to agents capable of arresting nuclear envelope breakdown and assays for use in discovering compounds capable of arresting the cell-cycle.

Fig. 1

Extract from *Xenopus* eggs provides a rich source of cellular material

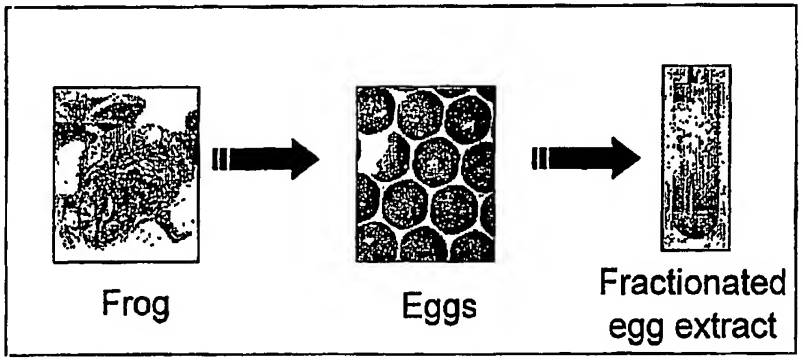

Fig. 2

Xenopus egg extract recapitulates nuclear assembly and disassembly events

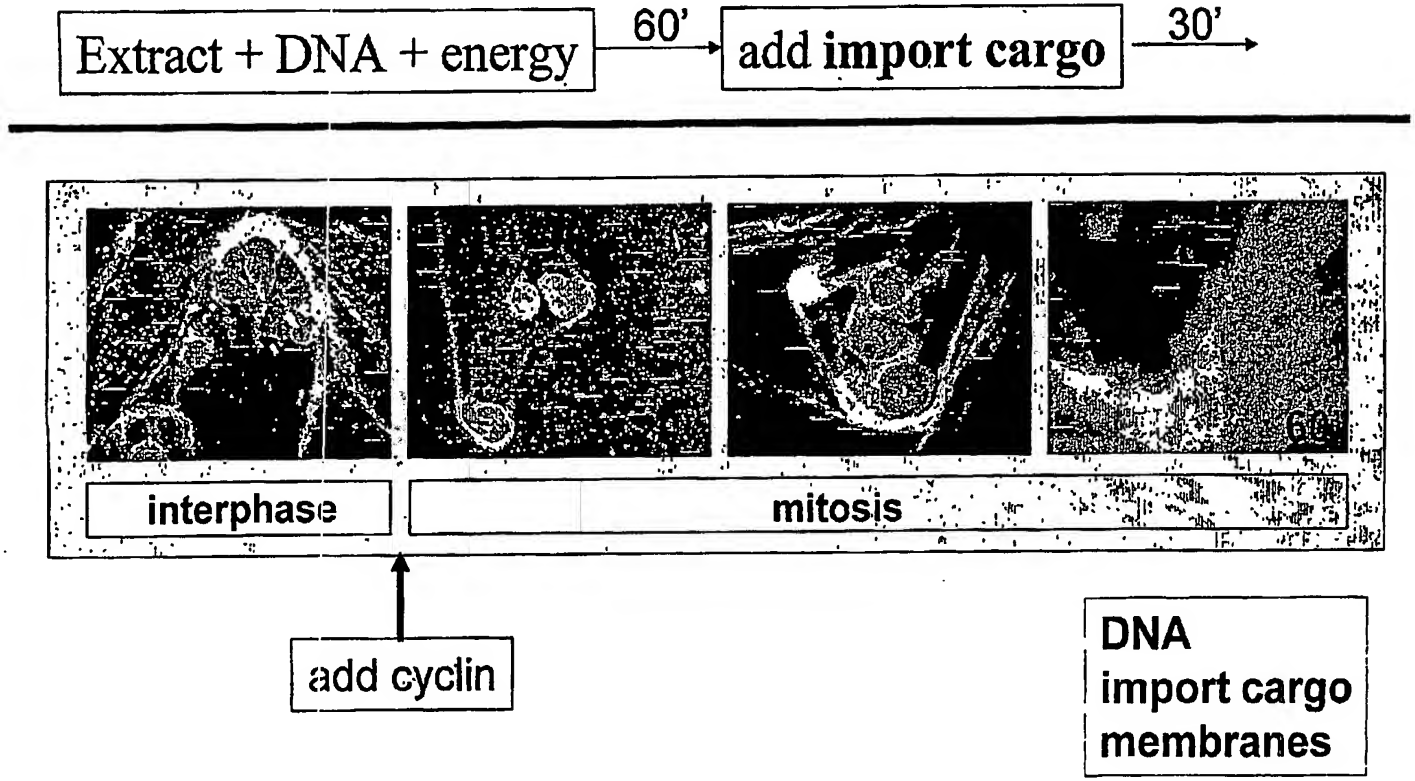

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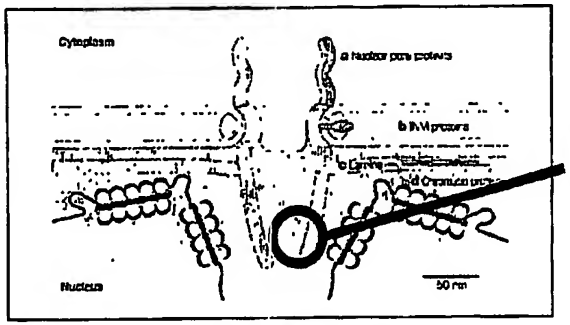
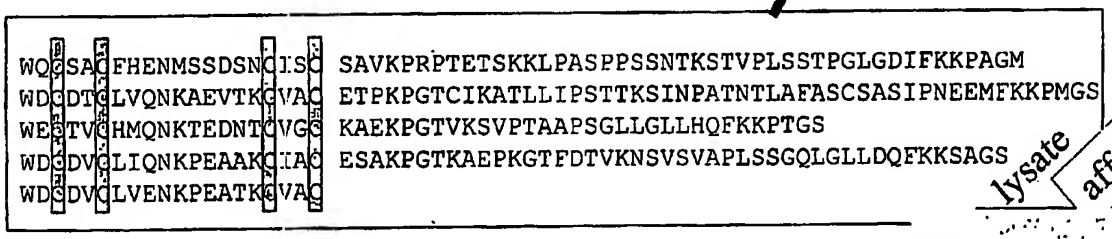
Fig. 3

Focus on Nup153 Zn finger domain: tools to probe function



component of the "pore basket"



express and purify
recombinant protein fragment

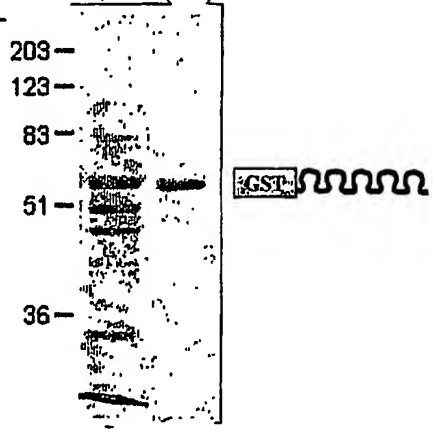
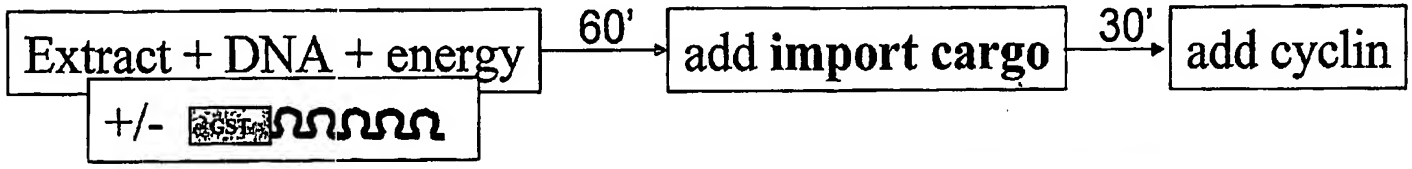
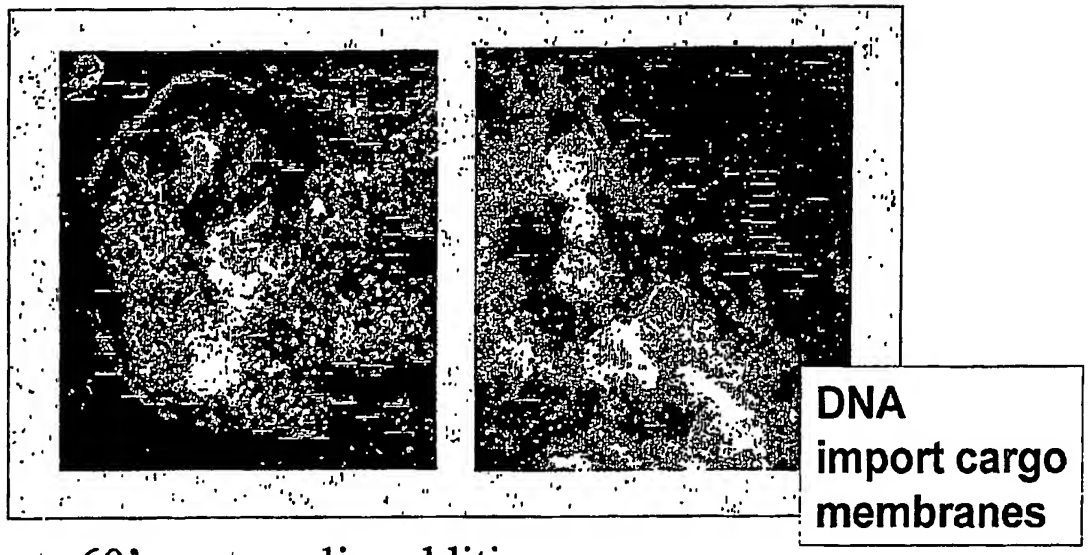


Fig. 4

Nup153 Zn Finger fragment interferes with nuclear disassembly





t=60' post cyclin addition

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Fig. 5

Nup153-specific antibodies

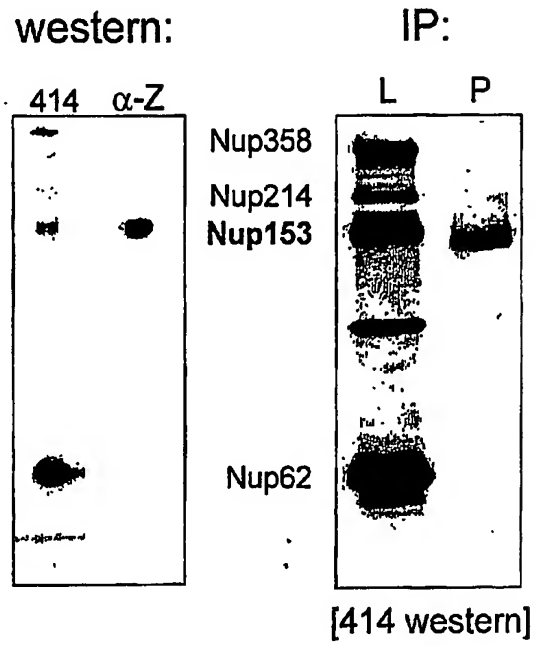
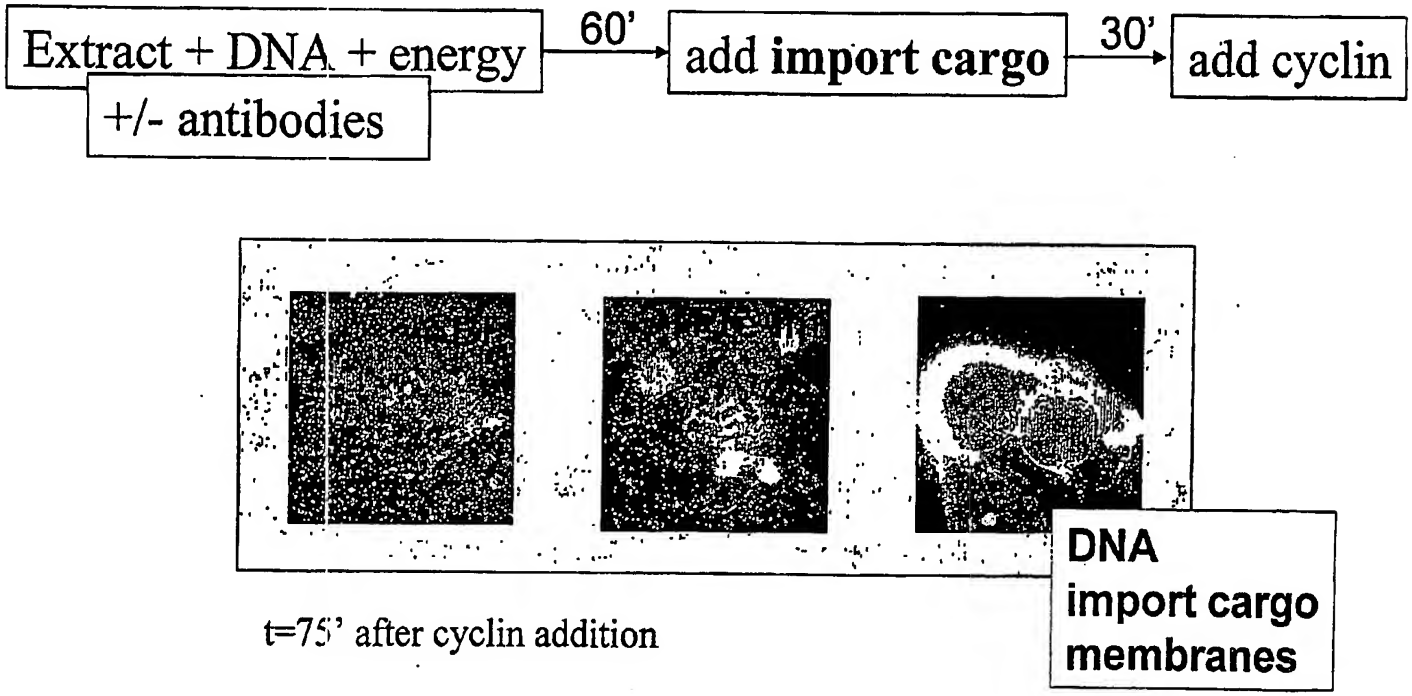


Fig. 6

Antibodies directed toward the Zn fingers of Nup153 block nuclear envelope breakdown



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